T cells (CTL). Due to the paucity of TIL extracted from CRC biopsies, functional interrogation ex vivo was done on TIL from melanoma biopsies and the authors found that *F. nucleatum* inhibited both CTL and $CD4^+$ T cells. *F. nucleatum* inhibited also IFN-γ production by memory $CD4^+$ T cells in the peripheral blood of HCMV-infected donors.

In view of such powerfully and widespread ability of some *F. nucleatum* strains to inhibit lymphocytes (although the link between TIGIT binding and hemagglutination is unclear), it is surprising that *F. nucleatum* activates NK cells in mice in a model of periodontitis. The NK cell receptor NKp46 directly recognized *F. nucleatum*, triggering NK cell production of TNF-α (Chaushu et al., 2012), a powerfully inflammatory cytokine that might lead to bone destruction in the pathogenesis of periodontitis, which in turn is also associated with APO, CVD, and RA. How can one reconcile the proinflammatory role of *F. nucleatum* in the periodontitis model with the immunosuppression in human lymphocytes? Fap2 does not bind mouse TIGIT, and therefore it might be impossible to untangle in vivo the intricacies of a potential ménage a trois among *F. nucleatum*, activating NKp46 and inhibitory TIGIT. It will also be important to test whether and how *F. nucleatum* and other commensals interact with innate lymphoid cells (ILCs) other than NK cells, because ILCs are clearly pivotal in regulating tissue homeostasis and barrier immunity in response to microenvironmental cues, including microbes.

While we mull over the chicken and egg question related to whether *F. nucleatum* is the cause or consequence of the conditions it is associated with, the work of Gur et al. provide a new molecular pathway that might help guide our thinking on ways to address the important question of how commensals turn into pathogens. Despite the fantastic therapeutic advance provided by immune checkpoint blockade, the response rate of cancer patients is still far from ideal. With improved microbial detection technology and due caution for potential contamination (Salter et al., 2014), cancer patients might be stratified according to the presence of bacteria in the tumor and, by targeting specific bacteria or the inhibitory immunoreceptors they interact with, one might add a weapon in the oncologists' arsenal. Dr. William Coley, considered one of the founding fathers of cancer immunotherapy (reviewed in Starnes C, 1992), might have found this approach interesting or dismissed it as a red herring.

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Mushrooming Insights into Skin Dendritic Cell Physiology

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Mechanisms responsible for protective immunity against epicutaneous *Candida* infections are incompletely characterized. In this issue of *Immunity*, Kashem et al. demonstrate that different *Candida* life forms engage selected skin dendritic cell subsets in distinct compartments, resulting in qualitatively different immune responses.

Candida albicans is a ubiquitous commensal fungus that colonizes human skin and mucosal surfaces and also causes infections. Mucocutaneous candidiasis is a common condition that occurs both in immune-competent and immunocompromised individuals. It has been established through genetic and immunological studies that interleukin-17 (IL-17)-mediated immunity is critical for protection against mucocutaneous candidiasis (Puel et al., 2010, 2011). Patients with inactivating mutations in IL-17 and the associated cytokine IL-6 develop a chronic and refractory form of candidiasis termed chronic mucocutaneous candidiasis (CMC) (Puel et al., 2011). Rare patients with APECED (autoimmune polyendocrinopathy candidiasis ectodermal dystrophy, also known as autoimmune polyendocrine syndrome) have neutralizing

autoantibodies against IL-17A, IL-17F, and IL-22 as a result of impaired tolerance caused by *AIRE* mutations (Kisand et al., 2010; Puel et al., 2010). As a consequence of impaired IL-17-associated immunity, APECED patients also develop CMC. Despite the susceptibility of individuals with IL-17 dysfunction to superficial *Candida* infections, impairment of this aspect of anti-fungal immunity does not routinely lead to invasive candidiasis.

Invasive candidiasis (manifested by organisms in visceral organs or the systemic circulation) is a life-threatening condition that typically occurs in immunocompromised individuals, including patients with AIDS and cancer and those receiving immunosuppressive agents for autoimmune and inflammatory diseases or subsequent to organ transplantation. Immune responses that protect against systemic candidal infections are not as well characterized as those that mitigate mucocutaneous candidiasis. Gain-of-function mutations in the STAT1 transcription factor gene lead to defective IL-17 and interferon-γ (IFN-γ) responses (Sampaio et al., 2013). This is associated with CMC and also disseminated coccidioidomycosis and histoplasmosis. Susceptibility to the latter two infections is attributed to defective T helper 1 (Th1) cell responses, suggesting that Th1 cell responses are important for protection against systemic fungal infections in general. Taken together, the literature suggests that protection against superficial and invasive fungal infections is conferred via different defense mechanisms.

Candida species are dimorphic fungi that grow as yeast at 30°C and as filamentous pseudo-hyphae at 37°C. When organisms from mucocutaneous candidiasis lesions are examined by microscopy, both yeast and pseudo-hyphae can be observed. Invasive *Candida* are exclusively pseudo-hyphae. The relative antigenicity and virulence of the two *Candida* life forms have not been extensively studied. Whether or not one *Candida* life form is more virulent or antigenic than the other has not been conclusively determined.

Skin harbors at least three dendritic cell (DC) subsets that are localized in two anatomically distinct compartments: Langerhans cells (LCs) in the avascular superficial epidermis and $CD103^+$ $CD11b^-$ and $CD11b^+$ DCs in the vascular underlying dermis. Despite extensive study, distinct in vivo functions of LCs have been difficult to demonstrate. Development of mouse models that allow selective depletion of the various skin DC subsets and carefully controlled studies of primary T lymphocyte responses to antigens whose distribution can be controlled has allowed for considerable progress in recent years (Heath and Carbone, 2013). For example, it is now clear that LCs survey the epidermis for antigens that have breached the stratum corneum while maintaining the epidermal tight junction barrier and subsequently elicit antigen-specific humoral responses (Ouchi et al., 2011) or T cell responses when the tight junction barrier has also been disrupted (Igyártó et al., 2011).

Kaplan and colleagues previously demonstrated that murine skin DCs promote different types of Th cell immune responses after epicutaneous *C. albicans* infection. LCs elicit Th17 cell responses, whereas $CD103^+$ dermal DCs promote Th1 cell responses. In this issue of *Immunity*, Kashem et al. (2015) go on to demonstrate that the nature of the *Candida* life form (yeast versus pseudo-hyphae) and associated distribution (epidermis versus dermis) are critical determinants that influence anti-*Candida* immune responses elicited by distinct skin DCs.

Epicutaneous infection with *C. albicans* is facilitated by mechanical disruption of the stratum corneum via depilation and sandpaper treatment prior to topical application of *C. albicans*. This protocol results in localized self-limited skin infections that are cleared within 1 week (Igyártó et al., 2011). Antigen-specific T helper cell responses were measured after epicutaneous infection with *C. albicans* strains that are genetically modified to express the peptide antigens 2W1S and Eα, either constitutively (in yeast and pseudo-hyphae) or exclusively in pseudo-hyphae. The authors confirmed that LCs were critical for Th17 cell responses and went on to demonstrate that IL-6 production by LCs was required. LCs upregulated IL-6 mRNA expression after epicutaneous *C. albicans* infection. Mice whose LCs were incapable of IL-6 synthesis failed to induce Th17 cell responses, although robust Th1 cell responses occurred (Figure 1). Dectin-1 is a C-type lectin that is expressed by dendritic cells and binds 1,3-beta-D-glucan. Protective Th17 cell responses developed only if *C. albicans* was in the yeast form and LCs expressed Dectin-1.

Dectin-1 has been reported to be critical for protection against systemic candidiasis (Marakalala et al., 2013). It is not obvious how to reconcile this observation with the concept that pseudo-hyphae that do not bind Dectin-1 cause invasive disease (Kashem et al., 2015). However, Kashem et al. (2015) did demonstrate that antigen-specific T cells proliferated in the absence of Dectin-1-*Candida* ligation and when *C. albicans* that are locked in pseudo-hyphae life forms were applied epicutaneously. Perhaps C-type lectins other than Dectin-1 recognize 1,3-beta-D-glucan, and ligation of these receptors initiates protective immune responses that have yet to be defined. Immune responses against *Candida* species are likely to be complex and redundant, potentially involving other pattern-associated molecular patterns (PAMPs), pattern-associated receptors (PARs), immune cells, and cytokines.

Although Th17 cells prevented epicutaneous *C. albicans* infection, they did not protect against systemic challenge. Rather, Th1 cell responses that did not require IL-6-producing LCs conferred protection against *C. albicans* that was introduced intravenously. One possibility is that IL-6-deficient LCs interact directly with T cells to efficiently induce Th1 cell responses. Alternatively, LC-derived IL-6 or Th17-cell-derived IL-17 might counterbalance immune responses that are induced by $CD103^+$ DCs, and loss of either (or both) of these cytokines results in prominent Th1 cell deviation (Figure 1). Kashem et al. (2015) did not determine whether similar Th1 cell deviation also occurs in Dectin-1-deficient mice or mice with Dectin-1-deficient LCs. If LC-derived IL-6 is required for Th17 cell development and lack of LC-derived IL-6 promotes Th1 cell skewing, LC-targeted immunization coupled with agonistic or antagonistic ligation of Dectin-1 could be utilized to modulate epicutaneous immunity. Alternatively, $CD103^+$ DCs could be selectively targeted to induce Th1 cell responses that might confer systemic immunity. The receptor that $CD103^+$ dermal DCs use to capture *C. albicans* remains to be identified.

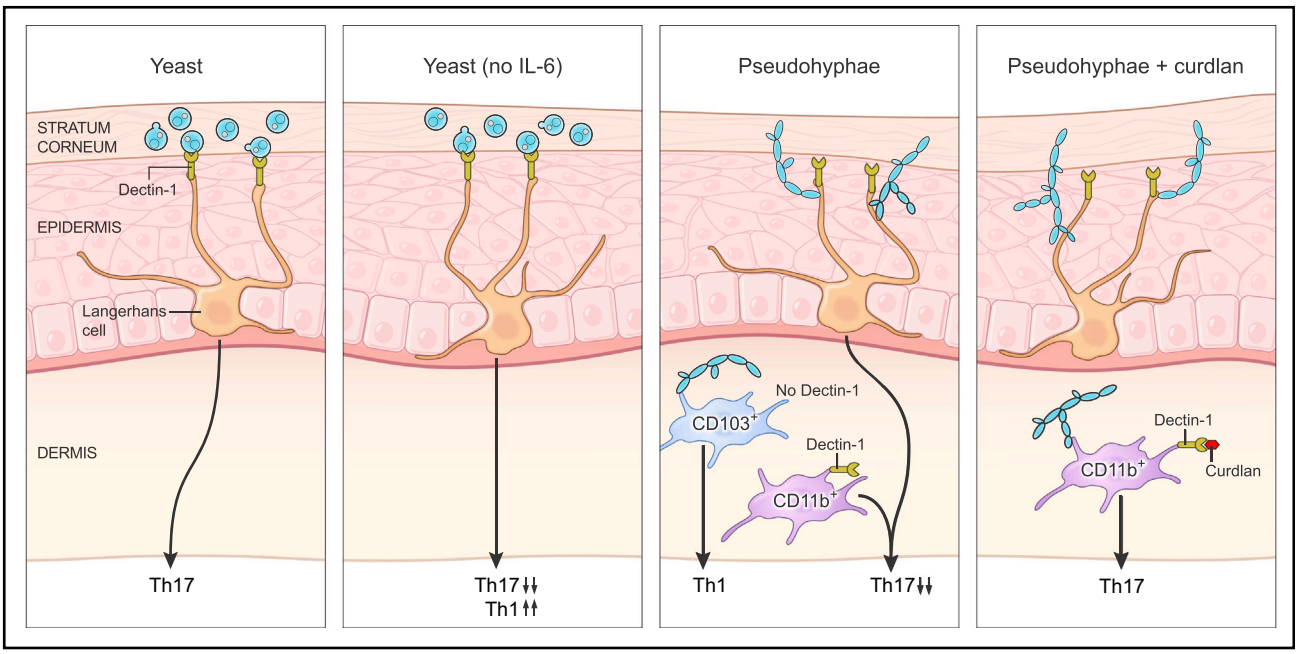

Figure 1. Different *Candida* Life Forms Engage Different Skin DCs to Effect Different Immune Outcomes
In this issue of *Immunity*, Kashem et al. (2015) demonstrate that epidermal Langerhans cells elicit Th17-cell-mediated immune responses against *Candida albicans* in its yeast form via an IL-6- and Dectin-1-dependent manner. Neither Langherhans cells or CD11b$^+$ dermal DCs respond to pseudo-hyphal life forms, but intradermal injection of *C. albicans* with the Dectin-1 agonist Curdlan enables CD11b$^+$ DCs to elicit Th17-cell-mediated responses. CD103$^+$ dermal DCs elicit Th1-cell-mediated anti-*Candida* immune responses via a mechanism that is independent of Dectin-1.

One of the experiments described in the present study suggests another potential vaccination strategy in which *Candida* could serve as an adjuvant. When genetically unmodified *C. albicans* was introduced epicutaneously in conjunction with LC-targeted protein antigen delivery accomplished via anti-Langerin antibody, mice exhibited robust antigen-specific Th1- and Th17-cell-mediated responses. It will be interesting to determine whether this approach is superior to conventional vaccines that are injected into skin. Although CD11b$^+$ DCs were unable to induce anti-*Candida* immune responses after intradermal injection of fungi, co-inoculation of Curdlan, a bacteria-derived form of 1,3-beta-D-glucan with infrequent 1,6-linkages, promoted Th17 cell responses. This indicates that CD11b$^+$ DCs can also be engaged to induce Th17 cell responses, representing yet another way that skin DCs might be manipulated to generate protective immunity.

Mucocutaneous candidiasis can be effectively treated with topical antifungals. However, invasive candidiasis in immunocompromised patients is difficult to treat and has high morbidity and mortality. Most *C. albicans* are sensitive to triazoles. However, drug-resistant *C. albicans* has become problematic over in recent years and several non-*albicans* pathogenic *Candida* species (including *C. glabrata* and *C. krusei*) are inherently resistant to triazoles (Maubon et al., 2014). Echinocandins, inhibitors of 1,3-beta-D-glucan (a cell wall component) synthesis, are utilized in these patients, but acquisition of resistance to echinocandins is also emerging (Maubon et al., 2014). Induction of adaptive immunity against 1,3-beta-D-glucan via vaccination would be an alternative to drug therapies and might be effective in treating patients who are infected with drug-resistant *Candida* species.

Kashem et al. (2015) provide experimental evidence that distinct Th cell responses protect against local and systemic *Candida* infections and that only yeast elicit local responses, providing a mechanistic link to human genetic diseases that exhibit susceptibility to local or systemic *Candida* infections. Although further information regarding *Candida* strain differences and receptors that recognize *Candida*-associated components will be important, this study provides insights that might facilitate development of new vaccination strategies to protect against local and systemic *Candida* infections. After confusion that has persisted for decades, the amount of definitive information regarding Langerhans cell function that we now have access to is "mushrooming" and an increasingly sharp picture is emerging.

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Immune Complexes: Not Just an Innocent Bystander in Chronic Viral Infection

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Understanding of how persistent viral infection impacts humoral immunity is incomplete. In this issue of *Immunity*, Wieland et al. (2015) and Yamada et al. (2015) find that high amounts of IgG-antigen complexes formed during chronic lymphocytic choriomeningitis infection can interfere with Fcγ-receptor-mediated effector activities, potentially contributing to immune dysfunction.

Immune complexes (ICs) typically form when polyclonal antibodies bind to polyvalent antigens forming large antibody-antigen complexes. While IC formation is an inevitable consequence of an effective immune response and is required for clearance of pathogenic antigens, inappropriate generation and deposition of such complexes results in pathology (Couser and Salant, 1980). Thus, the generation and clearance of immune complexes is a tightly regulated process; perturbations in immune complex clearance are associated with a variety of disease states due to activation of innate and adaptive immune cells within lymphoid organs or at a site of infection through crosslinking of Fcγ receptors (FcγR). In this issue of *Immunity*, papers by Weiland et al. and Yamada et al. describe a phenomenon whereby high amounts of IC formed during chronic lymphocytic choriomeningitis (LCMV) infection did not cause inflammatory disease due to crosslinking of FcγRs; instead, the IC were found to interfere with immunoglobulin G (IgG)-mediated effector functions by blocking FcγR interactions.

In the healthy host, IC production occurs in response to acute events such as infection, effectively restricting inflammatory signaling to instances warranting an active immune response. Cellular functions involved in clearing infection are, in part, mediated through FcγRs and include phagocytosis, antibody-dependent cell cytotoxicity (ADCC), antigen presentation, and selection of B cells during antibody responses (Pincetic et al., 2014). Following resolution of an infection, ICs are no longer generated and existing ICs are rapidly cleared from circulation.

In contrast, persistent IC formation occurs in a variety of chronic disease states and can result in unregulated, prolonged FcγR signaling when defects in IC clearance are also present. Chronic diseases with ICs are often of autoimmune, neoplastic, or infectious etiologies and occur when antibody is continuously produced, either appropriately (as in infection) or inappropriately (as in autoimmune and some neoplastic diseases), which reacts with pathogen or host antigens. In these settings, unchecked IC-FcγR interactions can culminate in hallmark sequelae of inflammatory diseases such as glomerulonephritis, vasculitis, arthritis, and bone erosion.

In addition to these classic inflammatory manifestations, persistent ICs can contribute to a broad range of abnormalities in innate and adaptive immunity, including elevated type 1 interferon (IFN) and dysregulated B and T cell functions. Elevated Type 1 IFN concentrations are sustained, in part, through TLR signaling following FcγR-mediated IC uptake; hyperactivation of immune cells can result, contributing to loss of peripheral tolerance, including increased autoantibody production as is classically seen in systemic lupus erythematosis, HIV, and in Lyme arthritis following *Borrelia burgdorferi* infection. Persistent elevation of type 1 IFN can also have broad immunosuppressive effects resulting from induction of the anti-inflammatory cytokine interleukin-10 (IL-10) and/or upregulation of programmed cell death ligand 1, an inhibitory coreceptor for T lymphocytes (Banchereau and Pascual, 2006). Persistent ICs are also associated with dysfunctional lymphocyte populations; B cell abnormalities are perhaps best characterized in humans in HIV infection and can include polyclonal activation (including of autoreactive specificities), suppressed antiviral IgA responses, and delayed or absent production of neutralizing antibodies (Moir and Fauci, 2009). T cell "exhaustion" in IC disease, characterized by loss of effector function, is present in several chronic human viral infections